

The dual kinase complex FAK-Src as a promising therapeutic target in cancer

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Abstract: Focal adhesion kinase (FAK) and steroid receptor coactivator (Src) are intracellular (nonreceptor) tyrosine kinases that physically and functionally interact to promote a variety of cellular responses. Plenty of reports have already suggested an additional central role for this complex in cancer through its ability to promote proliferation and anoikis resistance in tumor cells. An important role for the FAK/Src complex in tumor angiogenesis has also been established. Furthermore, FAK and Src have been associated with solid tumor metastasis through their ability to promote the epithelial mesenchymal transition. In fact, a strong correlation between increased FAK/Src expression/phosphorylation and the invasive phenotype in human tumors has been found. Additionally, an association for FAK/Src with resistances to the current anticancer therapies has already been established. Currently, novel anticancer agents that target FAK or Src are under development in a broad variety of solid tumors. In this article we will review the normal cellular functions of the FAK/Src complex as an effector of integrin and/or tyrosine kinase receptor signaling. We will also collect data about their role in cancer and we will summarize the most recent data from the FAK and Src inhibitors under clinical and preclinical development. Furthermore, the association of both these proteins with chemotherapy and hormonal therapy resistances, as a rationale for new combined therapeutic approaches with these novel agents, to abrogate treatment associated resistances, will also be reviewed.

Keywords: SRC, FAK, cancer, therapeutic target, FAK inhibitors, SRC inhibitors

The nonreceptor tyrosine kinases FAK and SRC The FAK-SRC complex in the integrin and tyrosine kinase receptor setting

Integrins are a family of transmembrane receptors that link the extracellular matrix (ECM) and the intracellular actin-cytoskeleton. These cell-matrix areas of adhesion are known as focal adhesion (FA) contacts/areas. Integrins cluster when they bind to ECM. Integrin clustering has a structural role but also induces the activation of intracellular signaling pathways that lead to important cellular responses such as proliferation, survival, migration and invasion in both normal and tumor cells.¹ In this setting, the linked activities of two nonreceptor intracellular tyrosine kinases, focal adhesion kinase (FAK) and steroid receptor coactivator (Src), is a common intracellular point of convergence in the signaling initiated by this integrin-ECM interaction. In response to the clustering, FAK associates to the cytoplasmic tail of the integrin and in response to this association FAK phosphorylates at its tyrosine residue 397 (Y397). Although this Y397 phosphorylation is mainly due to autophosphorylation; transphosphorylation by growth factors might also occur. This phosphorylated tyrosine provides a docking site for Src

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(Figure 1).² The tyrosine kinase Src, then, phosphorylates additional sites on FAK, leading to further increased activity of FAK and allowing the recruitment of proteins that contain Src homology 2 (SH2) domains such as Grb2 and PI3K.³ The mutually activated FAK/Src complex then initiates a cascade of phosphorylation events of new protein–protein interactions to trigger several signaling pathways that eventually leads to different cellular responses. For instance, Grb2, once bound to FAK can recruit SOS into the complex and activates the downstream Ras-MAPK pathway and/or may also transduces the signal through the activation of the PI3K-Akt cascade.^{3–5}

Adhesion is a hallmark of solid cancer cells and integrins are a nexus between intracellular signaling and cytoskeletal dynamics; besides, integrin-mediated signaling also intersects with growth factor-mediated signaling through various levels of cross-talk. In this complex network, the FAK/Src complex also transduces signals from tyrosine kinase receptors (TKRs) or integrates signals from both, integrins and TKRs, (see Figure 1) in normal and tumor cells.^{3,4} As an illustration of this connection in breast cancer, transforming growth factor β (TGF- β) has recently been found to be able to induce membrane-proximal clustering of human EGF (Epidermal Growth Factor) receptor 2, (HER2) and integrins by activating Src-FAK and receptor association to the cytoskeleton.⁶

Platelet-derived growth factor receptor (PDGF-R), epidermal growth factor receptor (EGF-R), fibroblast growth factor receptor (FGF-R), insulin-like growth factor-1 receptor (IGF-1R), hepatocyte growth factor (HGF-R), colony-stimulating factor-1 receptor (CSF-1R) or, stem cell factor receptor (SCF-R) are also TKRs that may transduce their signal by FAK/Src activation. As a consequence, the FAK/Src complex is potentially involved in different steps of tumorigenesis and further growth and metastatic progression of malignant tumors.^{7,8}

The increased expression or activity of FAK and/or Src in tumors is associated with a more invasive and aggressive phenotype and has led to the development of Src and FAK inhibitors as new anticancer drugs.^{9–12} These drugs are able to block proliferation, survival, angiogenesis and/or migration/invasion in preclinical tumor models¹³ and some of them have already shown preliminary antitumor activity in clinical trials with cancer patients.^{14–19}

FAK and Src structure

c-Src was the first characterized human oncogene. In 1909 Peyton Rous identified the Rous Sarcoma's Virus (RSV).^{20,21} Later, in 1958, the v-Src gene was identified as the cause

that allowed RSV to produce the sarcoma when the virus infected healthy chickens. The v-Src gene was taken up by RSV and incorporated into its genome conferring the virus the advantage of being able to stimulate uncontrolled proliferation in host chicken cells. Finally the human homologue of the v-Src gene, c-Src, was characterized as the first oncogene in humans.²²

c-Src is a nonreceptor tyrosine kinase. The Src family comprises of eight members in humans (Src, Fyn, Yes, Lyn, Lck, Hck, Blk and Fgr) with a molecular weight between 52–62 kDa.^{22,23} Each Src kinase family member is comprised of six domains. A SH4 domain placed at the N-terminal tail is involved in targeting Src to the plasmatic membrane. Adjacent to the SH4 domain, a region that is specific to each Src family member followed by a SH3 and a SH2 domain, both of them involved in the interaction of Src with other intracellular proteins (Figure 2).²³ Additionally, in the C-terminal tail, there is an SH1 domain involved in adenosine tri-phosphate (ATP) and substrate binding. This SH1 domain shows tyrosine kinase activity. The phosphorylation in the Y419 residue of the SH1 domain is required for maximum kinase activity. Immediately adjacent to the SH1 domain, there is another C-terminal region that acts as a negative regulatory domain that is itself regulated by phosphorylation. After phosphorylation of the Y530 residue, placed in this negative regulatory domain, Src undergoes conformational changes and becomes inactive.²³ Src activation is regulated at many different levels. In response to a signal stimuli Src translocates from the cytosol to the membrane where it will be activated by phosphorylation, the intracellular localization of Src is therefore one of the key regulatory mechanisms that control Src activation.²⁴ In addition the binding of FAK to the SH2 domain of Src relieves its autoinhibitory interaction that leads to the activation of Src. Once activated, Src phosphorylates FAK on a number of additional tyrosine residues, leading to further increased activity of FAK.^{2,23,25} In this sense, FAK acts as a molecular scaffold protein to activate and recruit Src to its substrates.

FAK was first identified in the search for proteins that were tyrosine phosphorylated in an integrin dependent manner and also in Src transformed fibroblasts as a key substrate of Src oncoprotein.²⁶ It was described as a focal adhesion-associated nonreceptor protein tyrosine kinase^{27,28} ubiquitously expressed and encoded by an evolutionarily highly conserved gene.

FAK harbors a central region with kinase activity that is flanked by a large N-terminal region that contains the erythrocyte band four 1-ezrin-radixin-moesin (FERM) domain and by a C-terminal region that contains the focal adhesion

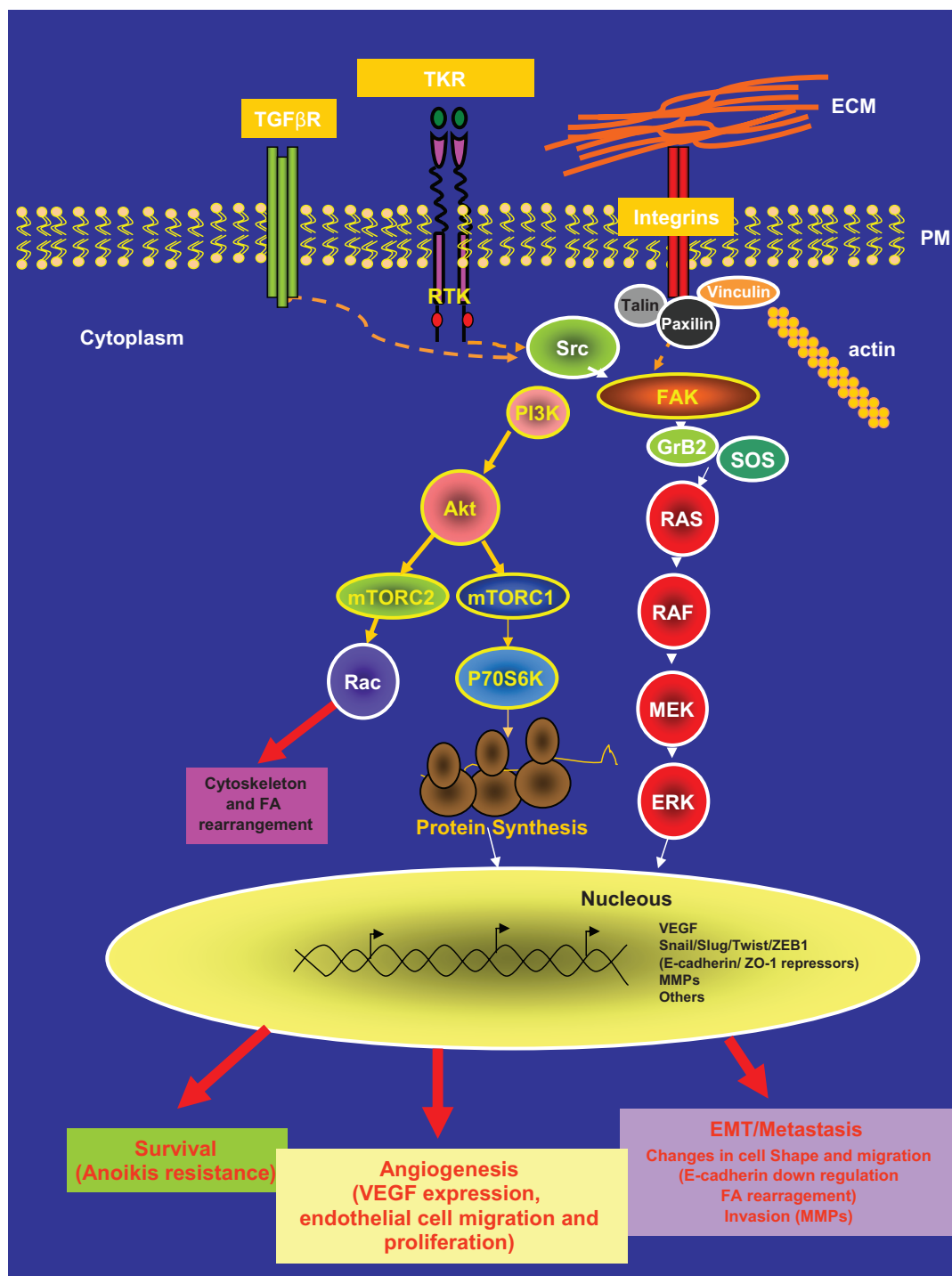


Figure 1 FAK/Src complex mediated signaling pathway.

Notes: The FAK/Src complex transduces signals from a variety of membrane receptors such as tyrosine kinase receptors and integrins through the activation of intracellular signaling pathways such as PI3K-Akt and Ras-MAPK to reach a cellular response.

Abbreviations: ERK, extracellular signal regulated kinase; FA, focal adhesions; MEK, mitogen-activated protein; FAK, focal adhesion kinase; VEGF, vascular endothelial growth factor; MMPs, matrix metalloproteinases.

targeting (FAT) domain. The Y397 residue, immediately adjacent to the kinase domain, is autophosphorylated in response to the clustering of integrins. This autophosphorylation increases the catalytic activity of FAK and creates a high affinity binding site for the SH2 domain of Src. This

interaction recruits and activates Src. The formation of the complex with Src is the most critical event in FAK-associated signaling. Src binds the Y397 residue and phosphorylates other FAK residues including Y576 and Y577 placed on the catalytic loop of the kinase and Y861 that are important for

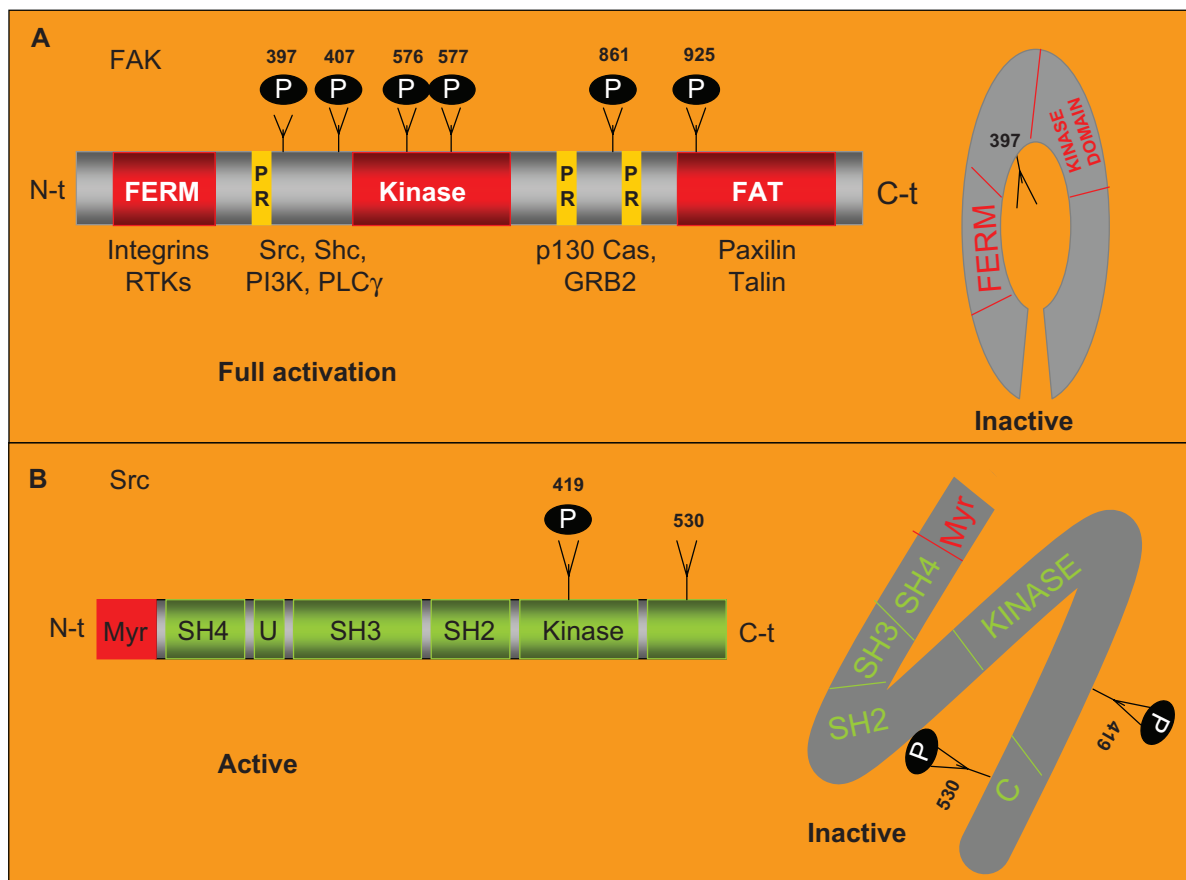


Figure 2 FAK and Src structure.

Notes: **A**) FAK structure. FAK harbors a central region with kinase activity that is flanked by a N-terminal region that contains the FERM domain and by a C-terminal region that contains the FAT domain. The autophosphorylation in its Y397 residue increases its catalytic kinase activity and allows the binding of specific intracellular proteins. FAK is phosphorylated by Src in its Y576 and Y577 residues allowing its full catalytic activity. In absence of stimulus, the FERM domain works as a negative regulator of FAK activity through its interaction with the kinase domain preventing the phosphorylation in Y397. In response of stimuli, the FERM domain interacts with the cytoplasmic tail of the integrins. Allowing the autophosphorylation of FAK in this tyrosine. The FAT domain, placed in the C-terminal region, mediates the co-localization of FAK with the FA areas through the interaction of FAK with the FA associated proteins talin and paxillin. Two proline-rich domains (PR) mediate the interaction of FAK with SH3 containing proteins such as p130 CAS. **B**) Src structure. Src is comprised of six domains: a SH4 involved in targeting Src to the plasmatic membrane, a region U that is specific of each Src family member, a SH3 and a SH2 domain involved in the interaction of Src with other intracellular proteins and a SH1 domain involved in ATP and substrate binding. The phosphorylation in Y419 residue of the SH1 domain is required for maximum kinase activity. Placed immediately adjacent to the SH1 domain there is a negative regulatory domain. After phosphorylation of the Y530 residue, in the negative regulatory domain, Src becomes inactive.

Abbreviations: FAK, focal adhesion protein; RTK, tyrosine kinase receptor; FERM, Band 4.1 Ezrin, Radixin, Moesin; PI3K, phosphoinositide 3 kinase.

full catalytic activity of FAK. Y925 has been also identified as an important site for phosphorylation by Src, which involves Src in induced epithelial mesenchymal transitions (EMTs).^{29,30} In the absence of stimuli from integrins and/or TKRs, the FERM domain works as a negative regulator of FAK activity. This domain interacts with the kinase domain preventing Y397 phosphorylation. Conversely, in response to co-clusters of integrins and TKRs, the FERM domain interacts with the cytoplasmic tail of the integrin allowing FAK-autophosphorylation in this Y397 residue. The FAT domain, placed in the C-terminal region, mediates the co-localization of FAK with the FA areas through the interaction of FAK with the FA associated proteins talin and paxillin. Furthermore, two proline rich domains, placed adjacent to the FAT domain, mediate the interaction of FAK with SH3

containing proteins such as p130CAS.^{9,11} The ability of FAK to promote the activation of p130CAS depends on Src activity. Although the scaffold function of FAK to recruit Src to its substrates is has already been well established, however less is known regarding FAK kinase activity.

Besides integrins, the N-terminal region of FAK also interacts with TKRs, and this interaction may be involved in the regulation of FAK activity and in addition in cross-talks between integrins and TKRs.³¹ Phosphorylation of FAK. Y861 promotes association of FAK with the $\alpha_v\beta_5$ integrin following vascular endothelial growth factor (VEGF) stimulation.³²

Different FAK isoforms from alternative splicing have been found: FAK, proline rich tyrosine kinase 2 (PYK2) and FAK related nonkinase (FRNK).⁹ FRNK lacks the catalytic

domain working as a FAK inhibitor competing with nontruncated endogenous FAK for the localization at FA areas.³³

FAK activity is under strict regulation by a variety of kinases and phosphatases such as glycogen synthetase kinase 3 type β (GSK3 β), tyrosine phosphatase SHP-2, serine/threonine protein phosphatase type 1 as well as by Src.^{9,34,35}

FAK and Src and their function in normal and tumor cells

Although, most available articles in the literature show FAK and Src as independent proteins, the current idea that both proteins work as a protein complex in the cellular signaling networks is emerging. FAK would form a binary complex with Src family kinases which can phosphorylate other substrates and trigger multiple intracellular signaling pathways that would induce different cellular responses.

FAK and Src are not only critical modulators of signaling pathways mediated by TKRs and integrins, they also respond to stimuli from G protein-coupled receptors, cell-cell adhesion proteins (ie, cadherins) and steroid hormone receptors to control a variety of normal and oncogenic cellular responses such as cell survival, proliferation and migration/invasion.^{4,22,23,34,36,37}

FAK-null embryos exhibit an early embryonic lethal phenotype.³⁸ These embryos show multiple defects, including a disorganized cardiovascular system due to extensive defects in angiogenesis and vasculogenesis.³⁸ Accordingly, over expression of FAK in vascular endothelial cells promotes angiogenesis.³⁹ In addition, conditional deletion of FAK in adult mouse epithelium was not lethal, and probably due to a functional compensatory effect mediated by its related family member PYK2.⁴⁰ Although PYK2 knock out mice develop normally except they do exhibit defective macrophage migration.^{41,42} The above data suggested that endothelial cells may possess an adaptive capacity to switch to PYK2 dependant signaling after deletion or inhibition of FAK. Thus, FAK and PYK2 inhibition may result in an antiangiogenic effect.

An interesting interaction has also been reported between FAK and the tumor suppressor protein p53, via the FERM domain that triggers p53 degradation, so that loss of FAK results in activation of p53 which could eventually suggest new approaches to trigger cytotoxic drug induced apoptosis.⁴³

Although, Src-null mice were viable, the analysis of homozygous mutants showed that they were deficient in bone remodeling (they had an impaired osteoclast function) and also developed osteopetrosis. This phenotype demonstrated that Src is not required for general cell viability

possibly due to a Src functional overlap with other related tyrosine kinases such as FAK. Therefore, Src may play an essential role in bone formation.⁴⁴ Accordingly, cancer patients treated with a Src inhibitor showed reduced serum levels of bone resorption markers suggesting Src inhibitors as a possible effective treatment for established bone metastasis.^{45,46}

In normal adult tissues and tumor cells, FAK and Src control many important biological processes within the cell. They have been associated with responses of cell growth and survival.^{9,10} In addition, they have also been involved in anoikis (apoptosis induced when anchorage-dependent cells detach from the surrounding ECM) resistance in tumor cells.⁴⁷⁻⁵⁰ Hence, FAK/Src activation may promote the anchorage-independent growth/transformation of tumor cells through the inhibition of the apoptotic response.^{48,49} FAK activation, in response to integrins, has an important role in FA turnover/rearrangement. This process is crucial for cell spreading and migration in physiological and pathological processes (ie, FA turnover is essential for EMTs during the metastatic behavior of tumor cells and in endothelial cell migration during tumor angiogenesis).⁹ Phosphorylation of FAK-Y925 is the major Src-specific phosphorylation event that is associated with integrin adhesion dynamics and E-cadherin deregulation during Src-induced EMT.^{29,30}

Furthermore, FAK has already been found, at elevated levels, in the majority of human cancers (head and neck, colon, breast, prostate, liver, thyroid, and others), particularly in highly invasive metastases.⁵¹ High levels of Phospho-FAK Y397 has already been found in: ovarian invasive tumors;⁵² acute myeloid leukemia;⁵³ squamous cell carcinoma of the larynx;⁵⁴ invasive cervical carcinoma;⁵⁵ invasive human colon cancer cells;⁵⁶ medullary thyroid cancer cell lines;^{57,58} human pancreatic cancer cells;⁵⁹ glioma cells;⁶⁰ and other tumor types. Furthermore, high levels of FAK phosphorylated in other tyrosine residues have already been found in specific tumor types. Papillary thyroid cancer samples show high phospho-Y861-FAK levels and high levels of phospho-Y861-FAK have also been correlated with sensitivity to the Src inhibitor AZD0530 in papillary and also in anaplastic thyroid cancer models.⁶¹ Although, the relationship between Src and cancer progression is best documented in colon and breast cancer.^{10,62} Src over expression or over activation has also been shown in a variety of human biopsies from primary tumors and their metastases (Figure 3).²³

An additional pro-angiogenic role for FAK and Src signaling in tumors has also been suggested.⁶³⁻⁶⁹ FAK

expression has been found in tumor endothelial cells from grade III and IV astrocytoma biopsies; whereas FAK expression was absent in endothelial cells of normal brain biopsies.⁶³ Accordingly, tumor endothelial cells transfected with FRNK (a negative FAK regulator) showed less migration *in vitro* than control cells; suggesting that FAK is involved in tumor-angiogenesis, at least in part, through the induction of endothelial cell migration.⁶³ Preclinical data from prostate cancer cell lines have also suggested a role for FAK signaling in the induction of VEGF expression in tumor cells.⁷⁰ Additionally, an intracellular cross-talk between the Ang-1 TKR (Tie-2) involved in angiogenesis in tumors and integrin pathways has also been shown.⁷¹ The binding of integrin $\alpha 1\beta 5$ to ECM-glycoproteins may lead to the association of Tie-2 with integrin $\alpha 1\beta$. The stimulation of Tie-2 by Ang-1 may promote the recruitment of FAK to the TKR-integrin areas inducing an endothelial cell response (sprouting and stabilization of the new tumor vessels).⁷¹ Src has also been associated with VEGF production in tumor cells.⁶⁶ In fact, Src inhibition decreases angiogenesis *in vivo*.^{68,69}

Immunohistological data about the expression/correlation between active FAK/Src on primary tumors and on their metastases is still awaited, to explore the value of FAK/Src as predictors of tumor outcome.

In fact, as we will review below, a link for FAK/Src with chemoresistance has already been reported in tumor models. In fact, FAK downregulation enhances docetaxel cytotoxicity in ovarian cancer cells.⁷² Moreover, FAK downregulation also increases gemcitabine chemosensitivity in pancreatic cancer cells.⁷³ Accordingly, a variety of reports show a role for Src in the promotion of chemoresistance.^{74–77} Src inhibitors have already shown single agent activity in cancer patients after their progression to chemotherapy.⁷⁸ Src inhibition promotes chemosensitivity in pancreatic cancer cells.⁷⁴ In addition, a combination of 5-fluorouracil (5-FU) and a Src inhibitor in 5-FU-resistant human pancreatic cancer cell lines restored 5-FU-induced apoptosis.⁷⁴ The potential mechanism for 5-FU chemosensitivity induced by Src inhibitors might be associated with the inhibition of the epidermal growth factor receptor-AKT (EGFR–AKT) pro-survival pathway induced by 5-FU. Fur-

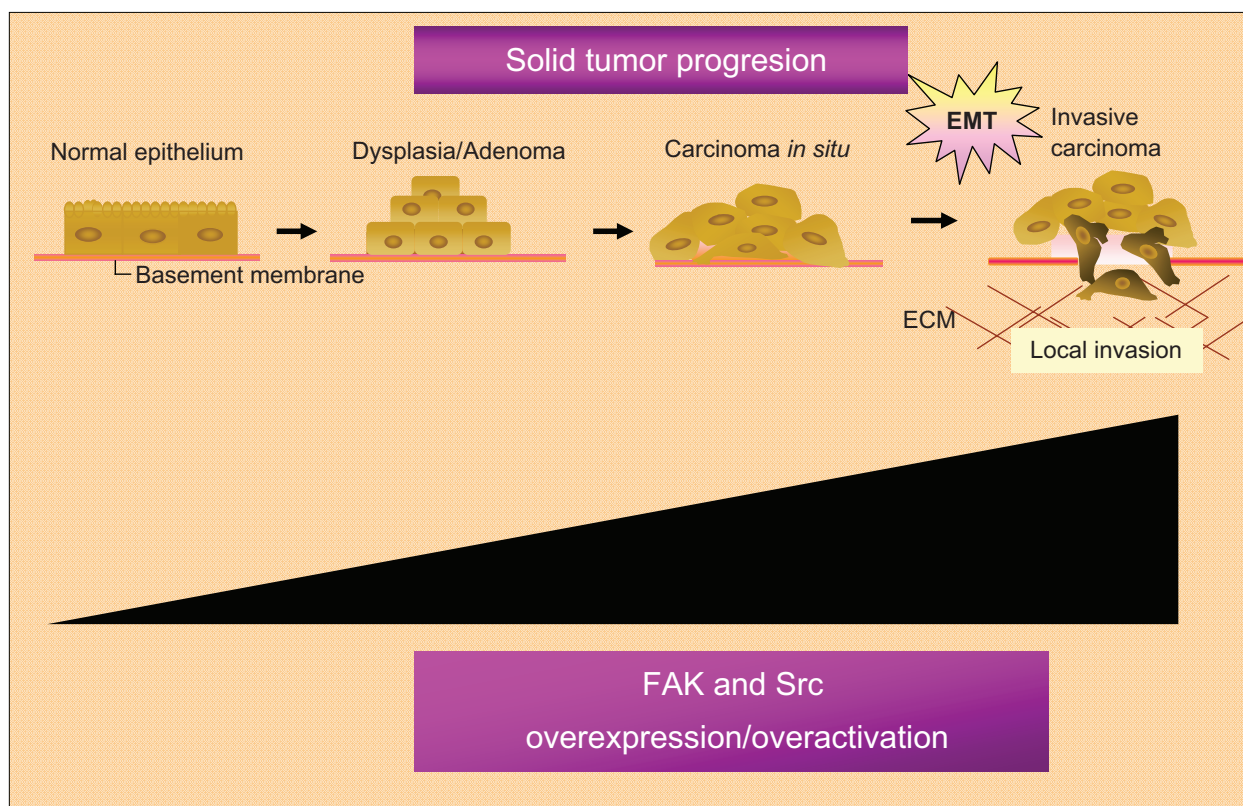


Figure 3 Role of the FAK-Src complex in the malignant progression of solid tumors.

Notes: FAK and Src play a critical role in solid tumor progression mainly through its ability to promote the epithelial-mesenchymal transition associated with the metastatic behavior of solid tumors.

Abbreviations: ECM, extracellular matrix; EMT, epithelial mesenchymal transitions; FAK, focal adhesion kinase; Src, steroid receptor coactivator.

thermore, a role for Src in mediating acquired endocrine resistance is also well established.¹⁰

Other FAK family members have also been associated with cancer. In fact, Pyk2 also shows a high expression and an association with tumor progression in a variety of tumor types such as: astrocytomas; breast; glioma; prostate; hepatocarcinoma; and nonsmall cell lung cancer.^{79–83} In addition, other Src family members have also been associated with solid and hematological tumors; and inhibitors against Src family members are under development as new anticancer drugs.⁸⁴

FAK-Src and tumor associated epithelial mesenchymal transition

Epithelial mesenchymal transition (EMT) is a complex of cellular and molecular processes by which epithelial cells acquire mesenchymal and migratory properties.⁸⁵ EMT takes place during critical phases of embryonic development and is also a crucial step in the infiltration and progression in solid tumors. Hallmarks of EMT include loss of cell–cell contacts, induction of FA turnover and increased expression of mesenchymal (fibronectin, vimentin, N-cadherin, α -smooth muscle actin, and others) and invasiveness (ie, metalloproteinases) markers.⁸⁶ The EMT is at the convergence of different molecular pathways involving cell survival and resistance to apoptosis, invasion and tumor angiogenesis, metastasis and drug resistance in advanced tumors.⁸⁷

A critical molecular feature in the loss of cell–cell contacts during EMT is the downregulation of the adhesion molecule E-cadherin (delocalization/loss of E-cadherin expression). A variety of membrane receptors such as integrins, TKRs, serine-threonine kinase receptors are able to induce E-cadherin downregulation during development and tumor progression through the activation of specific intracellular signaling cascades such as Ras-MAPK and PI3K-Akt-mTOR. In fact, transcriptional repressors (Snail, Slug, Twist, or ZEB1/2) involved in EMTs during development are also induced in response to EMT stimuli to repress E-cadherin expression during tumor progression.⁸⁶ Plenty of evidence suggests that FAK and Src, through its ability to integrate signals from numerous signaling receptors, plays a critical role in tumor-associated EMTs promoting intracellular signaling pathways that lead to the induction of E-cadherin repressors and to the subsequent E-cadherin downregulation as well as that promote FA turnover to allow tumor cell migration/invasion (Figure 1B).^{88–91}

New anticancer drugs that target FAK and Src

Based on evidence that supports FAK as a molecular scaffold protein, activated by Src to recruit its substrates; and that Src, as a tyrosine kinase is involved in the catalytic activation of FAK, and triggers FAK kinase activity to promote a variety of cellular responses during tumor progression, preclinical and clinical studies with new agents that employ different mechanisms for the blockade of FAK or Src kinases are currently underway.^{9,10} We show below a summary of the most advanced FAK and Src inhibitors under development (see Table 1).

Historically the first drugs synthesized with the aim of inhibiting T-cell activation via the Src family kinases Lck and Fyn were PP1 and PP2. The latest one is very selective for Src family kinases (SFKs). After PD173955 and PD173956 emerged with a lower selectivity than that of PP2, since these compounds were inhibitors of; Abl, Csk, platelet derived growth factor receptor (PDGFR) and EGFR. CGP76030 and CGP77675 were also multi-targeted agents against SFKs, Abl, EGFR and VEGF receptor (VEGFR) and SFKs, EGFR, FAK, and VEGFR, respectively. A third generation of molecules characterized by their higher potency in enzyme assays and their dual inhibition of c-Abl in a variety of imatinib-resistant c-Abl mutations appeared. The strong activity against c-Abl together with the potent anti-Src activity is explained by the strong structural similarity of the ATP binding domains in both kinases. The dual selectivity speeded up the development of these compounds.

At present, researchers are strongly focused on the study of the potential therapeutic benefits from the use of ATP-competitive kinase inhibitors against FAK and Src. These inhibitors interact with the ATP-binding pocket of FAK or Src and subsequently prevent FAK and Src autophosphorylation and therefore their activation.¹¹

Dasatinib

Dasatinib (BMS-354825) is an orally active small multiselective inhibitor, which inhibits the kinases Src and Abl with IC₅₀ values of 0.55 and 3.0 nM, respectively.⁹² Dasatinib also inhibits other Src family members such as Fyn (half maximal inhibitory concentration [IC₅₀] of 0.2 nM), LcK (IC₅₀ of 1.1 nM) and Yes (IC₅₀ of 0.4 nM).⁹² Dasatinib blocks the wild type chimeric Bcr-Abl protein which arise from Philadelphia chromosome.⁹³ Dasatinib is a 20-fold more potent inhibitor than imatinib in cells expressing wild-type Bcr–Abl hybrid protein and it also has an antitumoral effect in those tumor cells expressing Bcr–Abl imatinib-resistant mutants.⁹³ Dasatinib is also able to inhibit the tyrosine kinase receptors c-KIT, PDGFR- β

Table I Summary of FAK and Src inhibitors under clinical and preclinical development

| Drug | Target (IC50) | IC50 | IC50 | IC50 | Preclinical activity | Clinical activity |
|---------------------------|------------------|-----------------------|--------|----------------------|---|---|
| | Src | FAK | Abl | IGFIR | | |
| BMS-354825 (Dasatinib) | 0.55 nM | | 3.0 nM | | Solid and hematological tumor models | Approved in Imatinib refractory CML and Philadelphia chromosome + ALL, Phase I–2 clinical trials in solid and hematological tumors are underway |
| PF-562,271 | 797 nM | 1.5 nM (0.7 ng/mL) | | >500 nM | Colon, breast, prostate, pancreatic and lung tumor models | Phase I clinical trial in solid tumors has been already communicated |
| TAE-226 | | 100 nM/L = 100 nM | | 300 nM/L = 300 nM | Glioma and human pancreatic tumor cell lines | Not yet |
| AZD0530 | ≤4 nM 27 nM | | | | Skin, prostate, breast and pancreatic tumor models | Phase I–2 clinical trial underway |
| SKI-606 (Bosutinib) | 3.8 nM | | | | CML, colon and breast models | Phase I clinical trial has been already communicated, Ph II clinical trial in CML patients after imatinib failure under development |

Abbreviations: FAK, focal adhesion kinase; CML, chronic myelogenous leukemia; IGF, insulin-like growth factor; IC, inhibitory concentration.

and ephrins (EPHA2).⁹² Dasatinib has already shown to have broad preclinical activity in solid and hematological tumor models.^{14,92} It has already been approved by the Food and Drug Administration (FDA) and by the European Medicines Agency (EMA) for the second line treatment of imatinib-refractory chronic myelogenous leukemia (CML) and Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL).¹⁴ Currently, phase I/II clinical trials with dasatinib as a single agent or in combination are underway in a variety of solid and hematological tumors, based on the capability of dasatinib for blocking the activity Src family members.⁹⁴ Besides effectiveness in hematologic malignancies, dasatinib can suppress tumor growth in human breast cancer, human prostate, colon, ovarian, and lung cancer lines.^{95–100}

AZD0530

AZD0530 is another novel, orally administered, potent, and highly selective inhibitor of Src (IC50 value ≤ 4 nM), other Src family members like Lck and Yes (both with IC50 values < 4 nM) and Abl as well.¹⁰¹ Preclinical activity has been shown in: skin; breast; prostate; and pancreatic tumor models¹⁰¹ as well as estrogen receptor-positive breast cancer models¹⁰² where the combination of AZD0530 with tamoxifen¹⁰³ and aromatase inhibitors¹⁰⁴ prevented hormonal therapy resistance. This combination also shows an additive effect of delaying the growth of breast cancer cells.¹⁰⁵

A variety of phase I and II clinical trials are currently underway with AZD0530 in monotherapy or in combina-

tion, in patients with solid tumors based on its capability to inhibit Src.¹⁰⁶ Another possible use of AZD0530 has been recently reported. Based upon the hypothesis that Src could be involved in the development of metastases in xenograft models where the growth of the primary tumor was controlled by radiation; this report shows that those tumors from mice receiving AZD0530 regress more quickly following radiotherapy than their control counterparts.¹⁰⁷

Bosutinib

Bosutinib (SKI-606) is a potent, orally administered, bioavailable, dual Src (IC50 value of 3.8 nM) and Abl inhibitor which has already shown to have an antitumoral effect in chronic myelocytic leukemia (CML), colon, prostate and breast cancer models.^{108,109} Preclinical breast cancer models showed a decrease in *in vitro* cell motility and invasion and *in vivo* metastases after bosutinib treatment.¹¹⁰ A phase I clinical trial with bosutinib has been published showing; drug-related dose-limiting toxicity of grade 3 diarrhea and grade 3 rash (1 pt) with 400 mg being selected as the maximum tolerated dose.¹¹¹ Currently, phase II, proof of concept clinical trials, in patients with CML who had failed to improve with Imatinib, and in patients with solid tumors, are underway.¹¹²

PF-562,271

PF-562,271 is a potent ATP-competitive, small molecule inhibitor of both FAK and the related kinase Pyk2 (IC50 values of 1.5 nM (0.7 ng/mL) and 14 nM (7 ng/mL), respectively).

PF-00562271 also inhibits other kinases such as c-Src and insulin growth factor 1 receptor (IGF1R) with less selectivity (IC₅₀ value of 797 nM and IC₅₀ > 500 nM respectively). This inhibitor has shown a broad preclinical activity.¹¹³ In PC3 human prostate tumor cells, PF-00562271 treatment blocks, anchorage independent tumor cell growth and tumor cell migration *in vitro*, has shown antitumoral effects *in vivo*.¹¹ PF-00562271 decreases FAK phosphorylation-status *in vitro* and shows antitumor efficacy *in vivo*, in xenografts from: human colon; breast; prostate; pancreatic; and hepatocellular carcinoma tumor cell lines.^{113–115} No weight loss, or increase in morbidity and mortality were observed in any *in vivo* experiment and tumor growth inhibition was dose and drug exposure dependent. Furthermore, PF-562271 also showed an additional antiangiogenic effect over tumors.¹¹³ PF-562271 through the inhibition of FAK and PYK2 kinases may interfere with the ability of endothelial tumor cells to migrate, thus blocking the sprouting and stabilization of the new tumor vessels.^{63,116} PF-00562271 treatment has also led to the blockade of the expression of pro-angiogenic growth factors in tumor cells, such as VEGF.⁷⁰ Recently, Bagi and colleagues showed that PF-00562271 synergized with antiangiogenic agents that directly block VEGF signaling, through its ability to target different aspects of angiogenesis and tumor aggressiveness.¹¹⁵ In addition, these authors show that the combination of these agents not only led to the blockade of tumor growth, it also impacted upon the ability of the tumor to recover on withdrawal of the therapy. PF-562,271 has also showed an *in vivo* effect in preventing the loss of bone, suggesting its potential activity in patients with bone metastases and cancer-associated osteoporosis.¹¹⁷ Therefore, PF-562,271 may comprise of a combined action over tumors: antiproliferative; proapoptotic; antiangiogenic; and antimetastatic action. Based on the preclinical data, a dose escalation phase 1 clinical trial with PF-562,271, administered orally as a single agent, in patients with solid tumors is currently underway. Tumor responses with PF-562,271 have been already reached in ovarian, colon together with head and neck cancer patients. Preliminary results showed a manageable safety profile with PF-562,271. In addition, continuous oral dosing is feasible and may be extended over 6 to 12 months in the majority of patients. The most common adverse events in the 32 evaluated patients with monitored safety data was: nausea in 14 patients (<Grade 3); vomiting in 12 patients (only 1 patient with Grade ≥3); fatigue in 8 patients (<Grade 3); and diarrhea in 6 patients (<Grade 3). Prolonged disease stabilization has already been observed in a variety of solid tumors. However, the maximum tolerated dose (MTD) and recommended Phase 2 dose have still to be

published.^{15,16} In addition to PF 573,228, a closely related, early prototype FAK inhibitor¹¹⁸ has been reported to have appealing activity in combating ovarian cancer metastases,^{119,120} demonstrating the growing body of evidence that supporting research of Src/FAK inhibitors in epithelial carcinoma.

TAE 226

TAE 226 is a low molecular weight, ATP-competitive tyrosine kinase inhibitor of FAK and IGF1R with an IC₅₀ range of 100 to 300 nM/L.¹²¹ TAE 226 is still under preclinical development. Flow cytometry analysis of human glioma cell lines under TAE 226 treatment have shown an increase in the apoptotic and G₀ (quiescent/nonproliferative) fractions after treatment, when these cells were compared with control/nontreated cells.⁶⁰ TAE 226 induced-apoptosis in the glioma tumor model is mediated by caspases and is correlated with the p53 status. In fact, apoptosis was only induced in the subset of glioma cell lines containing the mutant p53 gene.⁶⁰

Additionally, TAE 226 treatment also prevented the *in vitro* attachment of these glioma cell lines. Furthermore, an *in vivo* intracranial glioma xenograft model showed a significantly higher median survival in the group of mice treated with TAE 226, at concentrations of 50–75 mg/kg. The treatment of ovarian cancer cell lines with TAE 226 inhibited cell growth in both a time- and dose-dependent manner; and enhanced docetaxel-mediated growth inhibition by 10 and 20 fold in the taxane-sensitive and taxane-resistant ovarian cell lines, respectively. In addition, TAE 226 alone and in combination with chemotherapy significantly prolonged survival in tumor-bearing mice. The efficacy of TAE 226 was related to: reduced pericyte coverage; the induction of apoptosis of tumor-associated endothelial cells; reduced microvessel density and tumor cell proliferation.¹²¹ TAE 226 also displayed an antitumoral effect in human pancreatic cell lines,¹²² esophageal cancer cell lines and xenografts, by a potent inhibition of PI3K-AKT-mTOR cell survival signaling.¹²³ Clinical trials with this new dual FAK-IGF1R inhibitor are planned. Recently, interesting experiments have suggested that the FAK-IGF-1R interaction site could be targeted; the specific disruption of this protein-protein interaction with another small molecule inhibitor (INT2-31) reinforces the potential novel role of this antineoplastic strategy.¹²⁴

FAK/Src and chemotherapy resistance

As we mentioned above, an association between FAK activation and resistance to chemotherapy has been broadly reported in human tumor models.^{72,73,125–128} Accordingly, the combination of conventional chemotherapeutic drugs with

FAK-targeting agents apparently offers greater efficacy in preclinical models than chemotherapy as a single agent. Treatment with FAK antisense oligonucleotides significantly induced apoptosis in human glioblastoma cells associated with a decrease in FAK protein levels.¹²⁹ The *in vitro* cytotoxic effect achieved with the anti-FAK agent in monotherapy was almost the same as those obtained with different chemotherapeutic regimens such as cisplatin, etoposide and nimustine hydrochloride.¹²⁹ When FAK antisense oligonucleotides and chemotherapy were administered in combination the antitumoral effect was clearly additive.¹²⁹ Treatment of squamous cell carcinoma models with recombinant FRNK peptides combined with etoposide, paclitaxel or 5-FU also showed an additive antitumoral effect.¹²⁷

The effect of combined chemotherapy and anti-FAK agents were also explored in human HCC cells *in vitro*.¹³⁰ When TNF- α plus cycloheximide was combined with FAK-antisense, an increase in the apoptotic index was observed.¹³⁰ Additionally, FAK siRNA was also able to potentiate gemcitabine action in pancreatic cancer cells⁷³ increasing the apoptotic index. The *in vivo* treatment with FAK siRNA, in combination with gemcitabine, induced in a statistically significant manner, a larger inhibition in the size of the tumors than gemcitabine in monotherapy.⁷³ FAK siRNA incorporated in liposomes was administered to mice bearing tumors from human ovarian cancer cells.¹³¹ Mice treated with siRNA-DOPC showed a decrease in tumor weight. Docetaxel in combination with siRNA-DOPC resulted in an even greater reduction in tumor weight.¹³¹ This combination also showed: antiangiogenic properties;¹³¹ it decreased microvessel density; VEGF and MMP-9 secretion; and increased apoptosis in tumor cells, in addition to tumor-associated endothelial cells.¹³¹ Treatment with siRNA-DOPC resulted in a decrease in the tumor weight of cisplatin-resistant xenografts as well.¹³¹ These data suggest that the combination of anti-FAK agents with docetaxel or cisplatin may be a valuable therapeutic approach in the chemotherapy of resistant ovarian cancer. Smith and colleagues showed that FAK downregulation enhanced the effects of 5-FU in human melanoma cells.¹³² FAK antisense oligonucleotides significantly increased cell detachment and apoptosis when they were administered alone or in combination with 5 FU.¹³² This led to the decrease in FAK protein levels, an effect that was also observed with the 5-FU alone. Accordingly, FAK blockade plus 5-FU showed an additive effect.

There are also, preclinical data supporting the role of Src in chemoresistance. The inhibition of Src reversed chemoresistance toward 5-FU in human pancreatic carcinoma cells.⁷⁴ Furthermore, Src inhibition also impaired both inherent

and acquired gemcitabine resistance in human pancreatic adenocarcinoma cells.⁷⁵ The combination of dasatinib and chemotherapy (5'-5'-DFUR or cisplatin) was synergistic in triple-negative breast cancer cells.⁹⁹ In addition, phase I clinical trials are currently ongoing, with the Src inhibitor dasatinib in combination with chemotherapy in solid tumors.^{94,133}

Interestingly the combination of paclitaxel/carbolatin standard chemotherapy in ovarian cancer has shown interesting synergism from this combined approach at the cell line level, that requires further investigation.¹³⁴

In conclusion, current evidence shows that FAK/Src-targeting compounds enhance the action of conventional anticancer agents at least in preclinical tumor models. Nonetheless, further molecular studies testing the activation status of both kinases in tumor biopsies and clinical trials, with anti-FAK/anti-Src agents and different chemotherapeutic schedules are still required to confirm if this complex is involved in treatment resistance and if the combination can enhance the efficacy of conventional chemotherapy in the clinical setting.

The interest of targeting FAK and Src in breast cancer

The Src/FAK signaling pathway is related to multiple receptor tyrosine kinases (RTKs) and intracellular mediators with a prominent role in the biology of the different subtypes of breast cancer.¹³⁵

On one hand, c-Src interacts with and contributes to the signaling cascade of different RTKs; modulates their turnover by interfering in the endocytosis; and ubiquitination; in taking part in the cytoskeleton rearrangement, migration and survival processes started at the RTKs' level in tumor cells.⁷ In breast cancer there is evidence of the interaction between c-Src and EGFR, (although short of a synergistic activity), connected to the crosstalk between estrogen receptor (ER) and EGFR.^{136,137} HER2 is coexpressed with c-Src in breast cancer¹³⁸ and their interaction seems to facilitate the migratory and metastatic phenotype of these cells.^{139,140} Likewise, HER2 is actually involved in c-Src regulation,¹⁴⁰ another mechanism of activation of HER2 such as the transactivation through G-protein receptors (ie, CXCR4).¹⁴¹ C-Src has also been proposed to be involved in the modulation of HER2-HER3 heterocomplexes in an intracellular mediated pattern.¹⁴²

However, Src has also been linked to the endoplasmic reticulum (ER) nongenomic activity¹⁴³ and the homeostasis of the ER¹⁴⁴ in the tumor cells.

Src inhibitors have been developed in different breast cancer subtypes. Triple negative breast cancer has emerged

as a potential field to be explored by this family of drugs. Two independent groups have described genetic profiles associated with a response to dasatinib^{95,145} highlighting the particular sensitivity of the basal-like phenotype to this Src inhibitor. This molecular marker approach has led to a phase 2 trial in the clinical setting¹⁴⁶ showing modest, although encouraging, results to be tested in further trials, in combination with chemotherapy.

Hormone receptor-positive breast cancer, resistant to the classic endocrine therapy strategies, has become another field actively studied. In the tamoxifen resistance setting, the upregulation of different RTKs' signaling pathways has been involved. It has been suggested that the resistant phenotype is not just the result of an estrogen independent growth,¹⁴⁷ but is also linked to an alteration in the relationship between the cells and the extracellular matrix, so that these tumor cells acquire an invasive and migratory phenotype^{148,102} that favors tumor dissemination. It has been demonstrated that anti-HER2 therapies are able to eliminate the agonist effect of tamoxifen, restoring its antitumoral capacity¹⁴⁹ and the blockade of both pathways, showing an increased efficacy against endocrine-resistant tumors.^{100,150,151}

However, these combinations have not shown a definitive effect regarding the migratory and invasive phenotype,¹⁴⁸ moreover, the tumoral cells eventually develop double resistance that results in an even more invasive behavioral pattern.^{102,152} This dual resistant phenotype is characterized by an increase Src kinase activity, that defines another opportunity to target endocrine resistant breast cancer. The *in vitro* utility of Src inhibitors; due to the Src/FAK relationship in the acquisition of endocrine resistance in breast cancer, has already been tested.^{153–156} However, a recent article showed an opposite role for Src in breast cancer. Campbell and colleagues analyzed 262 breast cancer specimens, before tamoxifen treatment, for active Src expression by tissue microarray. The authors showed that phosphorylated c-Src in the nucleus was significantly associated with improved patient outcome in ER-positive breast cancer.¹⁵⁷ The current findings suggest a crosstalk between ER and Src/FAK kinases, so that the addition of agents that block Src and/or FAK to hormonal therapy may improve the efficacy of the current endocrine therapies (aromatase inhibitors and tamoxifen). Clinical trials in breast cancer patients with dasatinib and aromatase inhibitors are underway in breast cancer patients.⁹⁴

Conclusions

As we reviewed above FAK and Src form a mutually activated complex that acts as a common intracellular point

of convergence in the signaling initiated by a variety of membrane receptors (RTKs, Integrins, G-coupled receptors, ER and others) to trigger a cascade of phosphorylation events and new protein–protein interactions in tumor cells and tumor endothelial cells, that allow the angiogenic and metastatic behavior of tumors. In fact, preclinical data with anti-Src and anti-FAK agents under development show that both types of inhibitors lead to antiproliferative, antiangiogenic and antimetastatic responses in human tumor models; a synergistic effect with other anticancer agents has been also observed. Therefore the inhibition of one of these kinases appears to be a successful therapeutic approach to avoid recurrence and dissemination of the primary tumor and also the progression of metastatic lesions.

Currently, we have robust data to believe FAK and c-Src inhibitors as a novel and promising anticancer strategy to combine with current anticancer therapies. A synergistic effect has already been shown when they are combined with other antitarget agents (ie, gefitinib, imatinib and sunitinib). Furthermore, these drugs have also been shown to be good candidates in the avoidance of chemotherapy and hormonotherapy resistances.

In addition, although it remains difficult to assess the efficacy of antimetastatic agents in the clinical setting, it appears that the inhibition of Src and FAK may have a potent anti-invasive effect, to delay tumor dissemination rather than real tumor shrinkage. Furthermore, although levels have already been seen as a possible predictor of response to Src inhibitors there is still a lack of suitable biomarkers that would be able to predict a response to these agents. In addition to current clinical studies of biomarker assays, the use of more sophisticated imaging technologies and the testing of the tumor, guided by biochemical rational, will help to maximize the development of these new compounds.

We have reviewed those trials with FAK and Src inhibitors under clinical development as a single agent or in combination with other therapeutic approaches. They have already shown clinical benefits in cancer patients with solid tumors. The identification of useful biomarkers to assess target inhibition, anti-invasive efficacy and predict treatment response will be crucial for future clinical trials.

Acknowledgments

We would like to thank to Nieves Ruíz-Ayllón for the editorial assistance. The authors report no conflicts of interest relevant to this research.

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